

# Is There an Association between Exposure to Environmental Estrogens and Breast Cancer?

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It was initially reported that levels of polychlorinated biphenyls (PCBs) or *p,p'*-DDE were elevated in breast cancer patients (serum or tissue) versus controls. These results, coupled with reports that selected environmental estrogens decreased  $17\beta$ -estradiol ( $E_2$ ) 2-hydroxylase activity and increased the ratio of  $16\alpha$ -hydroxyestrone/2-hydroxyestrone metabolites in MCF-7 human breast cancer cells, have led to the hypothesis that xenoestrogens are a preventable cause of breast cancer. More recent studies and analysis of organochlorine levels in breast cancer patients versus controls show that these contaminants are not elevated in the latter group. Moreover, occupational exposure to relatively high levels of PCBs and DDT/DDE are not associated with an increased incidence of breast cancer. A reexamination of the radiometric  $E_2$  2-hydroxylase assay in MCF-7 cells with diverse estrogens, antiestrogens, and carcinogens showed that the mammary carcinogen benzo[a]pyrene induced this response and the antiestrogen ICI 164,384 decreased  $E_2$  2-hydroxylase activity. Thus,  $E_2$  2-hydroxylase activity and the  $16\alpha$ -hydroxyestrone/2-hydroxyestrone metabolite ratio in MCF-7 cells does not predict xenoestrogens or mammary carcinogens. — *Environ Health Perspect* 105(Suppl 3):675–678 (1997)

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## Background

Several studies have shown that a number of industrial compounds exhibit estrogenic activity and these include various phenols and structurally diverse organochlorine environmental contaminants such as polychlorinated biphenyls (PCBs), hydroxylated PCBs, kepone, methoxychlor, DDT, DDE, and related compounds (1–7). Moreover, some of the persistent organochlorine environmental contaminants have been linked to reproductive failures in some wildlife species and in laboratory animal studies (8). These observations have led to the hypothesis that endocrine-disrupting environmental contaminants may be responsible for decreased male reproductive capacity in humans, including a possible worldwide decrease in male sperm counts over the past

50 years (9,10). Carlsen and co-workers (11) reported that metaanalysis of selected sperm count studies showed a precipitous decline and this was supported by reports from Paris, Sweden, and Scotland (12–14). However, recent studies have also reported that during the past 20 to 25 years, sperm counts in New York, Minnesota, California, Washington, and Toulouse, France, have remained constant (15–17). These results show remarkable regional variations in sperm counts with the current high values in New York being comparable to those previously reported in the 1950s through 1960s (18). These regional variations suggest that metaanalysis may not be appropriate for analysis of temporal trends in sperm counts; moreover, the variability in these trends suggests that decreased sperm counts may be a local/regional problem and not a global calamity. Another important component of the endocrine disruptor hypothesis is that xenoestrogens may also play a role in the observed increased incidence of breast cancer in the United States and many other countries (19,20). The Workshop on Hormones, Hormone Metabolism, and Breast Cancer focused on several aspects of this question and this paper will address issues raised by some of the invited speakers.

## Role of Organochlorine Xenoestrogens in Breast Cancer

Several studies have demonstrated that the overall cumulative exposure of women to estrogens results in an increased risk for breast cancer. Some of these risk factors include age at menarche, age at first birth, age at natural menopause, parity, and obesity (postmenopausal) (21). Dietary and lifestyle factors also play a role in development of breast cancer and this is supported, in part, by the increased incidence of this disease in Oriental women (low incidence) who migrate to the United States. Davis and co-workers (19,20) have also hypothesized that changes in exposures to xenoestrogenic substances may partly account for recent trends in breast cancer.

The potential role of organochlorine compounds as etiologic agents in breast cancer was suggested by results of case-control studies that showed that *a*) levels of DDT and related metabolites (primarily *p,p'*-DDE) were higher in serum or tumors of breast cancer patients versus controls (22); *b*) PCB levels were higher in mammary adipose tissue of breast cancer patients in Connecticut than in controls (23); *c*) DDE levels were higher in mammary adipose tissue of estrogen receptor-positive breast cancer patients compared to controls (24); and *d*)  $\beta$ -hexachlorocyclohexane levels in tumors were higher than in controls (25). All of these studies contained 58 or fewer individuals in their patient groups. Krieger and co-workers (26) used a cohort of 150 breast cancer patients and 150 control subjects in California and showed that there was not a significant difference in serum organochlorine levels in the two groups. There are several ongoing studies that are investigating levels of organochlorine compounds in breast cancer patients and control groups; however, at present the following points should be considered:

- Analysis of all the available data indicates that organochlorine levels are not significantly higher in breast cancer patients than in controls (27–29). In their review of the data for breast and endometrial cancers, Adami and co-workers (29) stated that “available data do not indicate that organochlorines will affect the risk of these two cancers in any but the most unusual situation.” The overall ratio of means (cases/controls) for DDE and PCBs in all the

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Abbreviations used: DMBA, dimethylbenz[a]anthracene;  $E_1$ , estrone;  $E_2$ ,  $17\beta$ -estradiol; ER, estrogen receptor; I3C, indole-3-carbinol; PCBs, polychlorinated biphenyls.

studies were 1.08 and 1.03 and were not significantly elevated (29).

- Adami and co-workers (29) also reviewed the breast cancer incidence in women occupationally exposed to PCBs; their summary analysis reported an overall observed/expected ratio of 0.84, indicating that among women highly exposed to PCBs, there was not an increased incidence of breast cancer. Moreover, high exposure to DDT has not been associated with an increased risk of breast cancer (28,29).
- Wolff (30) recently pointed out that adipose tissue levels of DDT and related metabolites (and PCBs) have been declining since 1965 and similar decreases in breast milk DDE levels have been reported in Scandinavian countries (31). These declines correspond to the restricted use and ultimate ban of DDT in most industrialized countries, even though there is continued use of this pesticide for insect control in less-developed nations. Nevertheless, the marked decline in DDE levels and the parallel increased incidence of breast cancer does not support an etiologic role for DDT in this disease.
- Several studies have demonstrated the estrogenic activity of DDT and related compounds as well as some PCBs and hydroxylated PCBs in breast cancer cells and in the rodent uterus. However, it is also true that other persistent chlorinated pollutants including some PCB congeners, polychlorinated dibenzofurans and dibenzo-*p*-dioxins, exhibit antiestrogenic activities in the same bioassay (32). Safe (33) has suggested that in terms of dietary intake of organochlorine pollutants, the overall intake of antiestrogen equivalents was greater than estrogen equivalents in the diet. This is an issue that should be further investigated. In their article hypothesizing that xenoestrogens may play a role in breast cancer, Davis and co-workers (19) pointed out that DDT accelerated 2-acetamidophenanthrene-induced mammary gland tumors in the male Sprague-Dawley rat (34). This is not a widely used animal model or carcinogen for investigating development and growth of breast cancer in women. In contrast, they failed to point out a study by Silinskas and Okey (35), who used the 7,12-dimethylbenz[*a*]anthracene (DMBA) model to show that DDT protected against mammary tumor formation in female rats.

These results do not support the hypothesis that organochlorine xenoestrogens are etiologic agents for breast cancer. However, the studies that have reported elevated levels of PCBs, DDE, and polybrominated biphenyls (36) in breast cancer patients should be further investigated. Persistent halogenated aromatic compounds preferentially bioconcentrate in fatty tissue and these compounds may be a biomarker of important dietary factors that play a role in mammary carcinogenesis.

### Good and Bad Estrogens?

Bradlow and co-workers (19,20,37) have hypothesized a possible role for the 2-hydroxy and 16 $\alpha$ -hydroxy metabolites of 17 $\beta$ -estradiol ( $E_2$ ) and estrone ( $E_1$ ) in the development of breast cancer. 16 $\alpha$ -Hydroxy- $E_1$  levels were elevated in strains of mice susceptible to mammary cancer and levels were also elevated in breast cancer patients (38,39). Osborne and co-workers (40) also reported that  $E_2$  16 $\alpha$ -hydroxylase activity was higher in mammary terminal duct lobular units from breast cancer patients than in patients undergoing reductive mammoplasty. Swaneck and Fishman (41) also reported that 16 $\alpha$ -hydroxy- $E_1$  covalently binds to the estrogen receptor (ER) in MCF-7 cells and suggested that this may play a role in malignant transformation of mammary tissue. In contrast, increased levels of  $E_2$  2-hydroxylase activity are associated with protection from mammary cancer in both animal models and in humans; moreover, 2-hydroxy- $E_2$  is elevated in animal models and humans exposed to indole-3-carbinol (I3C) and related compounds that are protective against mammary cancer (38,41–43). The concept of good (2-hydroxy- $E_1$ / $E_2$ ) and bad (16 $\alpha$ -hydroxy- $E_1$ / $E_2$ ) estrogens was recently expanded by Bradlow and co-workers (37), who proposed that the 16 $\alpha$ -hydroxy- $E_1$ /2-hydroxy- $E_1$  metabolite ratio in MCF-7 cells treated with  $E_2$  (radiolabeled with [ $^3$ H] at C-2 or C-16) was predictive for mammary carcinogenesis. The compounds used in their study included the following: I3C, an anticarcinogen; DMBA, a mammary carcinogen; atrazine, a putative estrogen that enhances time-to-tumor formation in one rat strain; and several organochlorine pesticides that were either weakly estrogenic or indirectly associated with elevated breast cancer levels in some studies ( $\gamma$ -hexachlorocyclohexane, 2,2',4,4',5-pentachlorobiphenyl, kepone, *p,p'*-DDE, *p,p'*-DDT, *o,p'*-DDE, *o,p'*-DDT, endosulfan-1, endosulfan-2, and

*p,p'*-DDethane). Treatment of MCF-7 cells with 10  $\mu$ M I3C increased 2-hydroxylation and decreased 16 $\alpha$ -hydroxylation of  $E_2$ , whereas DMBA, atrazine, and the organochlorine pesticides decreased  $E_2$  2-hydroxylase and increased 16 $\alpha$ -hydroxylase activities using a radiometric assay procedure. The predictive value of these hydroxylase activities and their ratios presented some problems based on results of other studies and these are indicated below.

- The induction of CYP1A1-dependent  $E_2$  2-hydroxylase activity by 10  $\mu$ M I3C is inconsistent with previous studies, which report that I3C induces CYP1A1 gene expression or immunoreactive protein at concentrations > 125  $\mu$ M (44,45).
- Recent studies in this laboratory show that 10  $\mu$ M I3C acts as a partial aryl hydrocarbon receptor antagonist (45).
- Most of the organochlorine pesticides included in this study are not active as inducers in cancer cell lines and their activity as inducers or inhibitors of  $E_2$  16 $\alpha$ - or 2-hydroxylase activity has not previously been reported.

Atrazine was included in the list of compounds due to its designation as an estrogenic compound (20); however, recent studies have shown that this compound is not estrogenic in several diagnostic bioassays including the E-SCREEN (7,46). Therefore, research in this laboratory (47) has reinvestigated the effects of several model compounds as inducers of  $E_2$  2-hydroxylase activity in MCF-7 cells using the radiometric assay. The results obtained for I3C were similar to those reported by Bradlow and co-workers (37); namely, a 2- to 4-fold induction of  $E_2$  2-hydroxylase was observed 48 hr after treatment with I3C. However,  $E_2$  2-hydroxylase activity was also induced after treatment with I3C for only 2 hr, indicating that the induced response was due, in part, to an *in vitro* effect and not an induction response. Similar results were obtained for atrazine and DMBA which caused decreased  $E_2$  2-hydroxylation in MCF-7 cells after incubation for 2 or 48 hr. Moreover, it was also shown that benzo[*a*]pyrene, a mammary carcinogen, induced  $E_2$  2-hydroxylase activity and this was consistent with previous reports showing that this compound induced CYP1A1-dependent activity in MCF-7 cells (48). In contrast, two potent antiestrogens, ICI 164,384 and ICI 182,780, decreased  $E_2$  2-hydroxylase activity in MCF-7 cells after incubation for 48 hr. These results indicate that a mammary carcinogen can induce  $E_2$  2-hydroxylase activity whereas this response

is inhibited by some antiestrogens that are anticarcinogens.

## Summary

Although overall exposure to estrogens is a known risk factor for development of breast cancer, current data do not support a role of organochlorine compounds such as PCBs and DDE as etiologic agents. It is possible

that there are other unknown sources of human exposure to xenoestrogens; however, their potential impact must be considered along with relatively high dietary intakes of natural estrogenic (e.g., bioflavonoids) and antiestrogenic (e.g., I3C) substances in food (33). The results of recent studies in the laboratory suggest that the radiometric  $E_2$  2-hydroxylase activity in MCF-7 cells is not

a consistent predictor of mammary carcinogens; however, this does not preclude the use of the  $16\alpha$ -hydroxy- $E_1/2$ -hydroxy- $E_1$  ratio for predicting cancer risk in other *in vitro* or *in vivo* bioassays. The significance of  $16\alpha$ - and 2-hydroxy- $E_2/E_1$  metabolite ratios as prognostic indicators for mammary cancer is controversial (49) and also requires further investigation.

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